Current Biology, Vol. 15, 2243–2248, December 20, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.10.055

Report

# Identification of the Minimal Protein Domain Required for Priming Activity of Munc13-1

David R. Stevens,[1,3] Zheng-Xing Wu,[1,3] Ulf Matti,[1] Harald J. Junge,[2,4] Claudia Schirra,[1] Ute Becherer,[1] Sonja M. Wojcik,[2] Nils Brose,[2,*] and Jens Rettig[1,*]
[1]Physiologisches Institut
Universität des Saarlandes
Kirrberger Str. 8
D-66421 Homburg/Saar
Germany
[2]Abteilung Molekulare Neurobiologie
Max-Planck-Institut für Experimentelle Medizin
D-37075 Göttingen
Germany

## Summary

**Most nerve cells communicate with each other through synaptic transmission at chemical synapses. The regulated exocytosis of neurotransmitters, hormones, and peptides occurs at specialized membrane areas through $Ca^{2+}$-triggered fusion of secretory vesicles with the plasma membrane [1–7]. Prior to fusion, vesicles are docked at the plasma membrane and must then be rendered fusion-competent through a process called priming. The molecular mechanism underlying this priming process is most likely the formation of the SNARE complex consisting of Syntaxin 1, SNAP-25, and Synaptobrevin 2. Members of the Munc13 protein family consisting of Munc13-1, -2, -3, and -4 were found to be absolutely required for this priming process [8–13]. In the present study, we identified the minimal Munc13-1 domain that is responsible for its priming activity. Using Munc13-1 deletion constructs in an electrophysiological gain-of-function assay of chromaffin-granule secretion, we show that priming activity is mediated by the C-terminal residues 1100–1735 of Munc13-1, which contains both Munc13-homology domains and the C-terminal $C_2$ domain. Priming by Munc13-1 appears to require its interaction with Syntaxin 1 because point mutants that do not bind Syntaxin 1 do not prime chromaffin granules.**

## Results

In order to identify the minimal region of Munc13-1 required for its priming activity, we generated deletion constructs of Munc13-1 and tested their priming function in a gain-of-function assay by measuring exocytosis from mouse adrenal chromaffin cells. Chromaffin cells are particularly well suited for this approach for two reasons. First, the endogenous expression level of Munc13-1 is very low [14], and Munc13-1-deficient chromaffin cells have normal secretion. Therefore, it is possible to perform studies on the function of Munc13-1 in the wild-type background. Second, overexpression of full-length Munc13-1 in bovine [14] and mouse chromaffin cells (see Figure S1 in the Supplemental Data available with this article online) leads to a 3-fold increase in $Ca^{2+}$-triggered catecholamine release, an increase that is due to an increased priming rate as indicated by an unaltered number of morphologically docked vesicles [14]. Because even a construct lacking the first 519 amino acids, Munc13-1(520–1735), still retains its full priming activity in adrenal chromaffin cells [15], we focused our analysis on the C-terminal two-thirds of Munc13-1 (Figure 1). For identification of infected cells, a green fluorescent protein (EGFP) was fused as a fluorescent marker to the C termini of all constructs. After generation of Semliki-Forest viruses, we tested the expression of the different constructs in cultured kidney cells by western-blot analysis. As shown in Figure S2, all fusion constructs were expressed at the expected molecular size. Only the Munc13-1(792–1735) construct showed a second band at 130 kDa, which most likely represents a breakdown product.

To test the priming activity of the Munc13-1 deletion constructs, we overexpressed them in adrenal chromaffin cells, which represent one of the best-characterized model systems for the study of regulated secretion [2, 3]. After establishing whole-cell configuration, we first stimulated chromaffin cells with a voltage protocol consisting of seven 100 ms depolarizations at 10 Hz. The resulting membrane capacitance ($\Delta C_m$) increases due to $Ca^{2+}$-dependent exocytosis were monitored by the patch pipet and compared to noninfected chromaffin cells, which served as controls. As shown in Figure 2A, overexpression of a construct encoding the C-terminal two-thirds of Munc13-1 [Munc13-1(644–1735)-EGFP] led to an average capacitance response of 662 ± 110 fF (n = 16). In contrast, the average capacitance increase in control cells was only 198 ± 28 fF (n = 12, $p < 0.01$). This increase in secretion was comparable to the effects of overexpression of full-length Munc13-1 ([14] and Figure S1) and indicates that the C-terminal two-thirds of the protein are sufficient to produce this gain-of-function effect. The gain-of-function effect on priming was also observed upon further deletion of the second $C_2$ domain [518 ± 70 fF, n = 21, Munc13-1(792–1735)-EGFP versus 295 ± 42 fF, n = 15, $p < 0.001$, control; Figure 2B], and deletion of the region connecting the second $C_2$ domain and the first MHD [521 ± 70 fF, n = 13, Munc13-1(1100–1735)-EGFP versus 297 ± 67 fF, n = 15, $p < 0.001$, control; Figure 2C]. In contrast, further deletion of the first MHD or both MHDs resulted in capacitance responses indistinguishable from those of control cells [87 ± 25 fF, n = 13, Munc13-1(1332–1735)-EGFP versus 101 ± 32 fF, n = 11, control; Figure 2D; 294 ± 66 fF, n = 9, and Munc13-1(1551–1735)-EGFP versus 302 ± 42 fF, n = 12, control; Figure 2E]. These data indicate that the presence of the MHDs of Munc13-1 is necessary for its priming

*Correspondence: brose@em.mpg.de (N.B.); jrettig@uniklinik-saarland.de (J.R.)
[3]These authors contributed equally to this work.
[4]Present Address: Department of Tumor Biology and Angiogenesis, Genentech Inc., South San Francisco, California 94080.

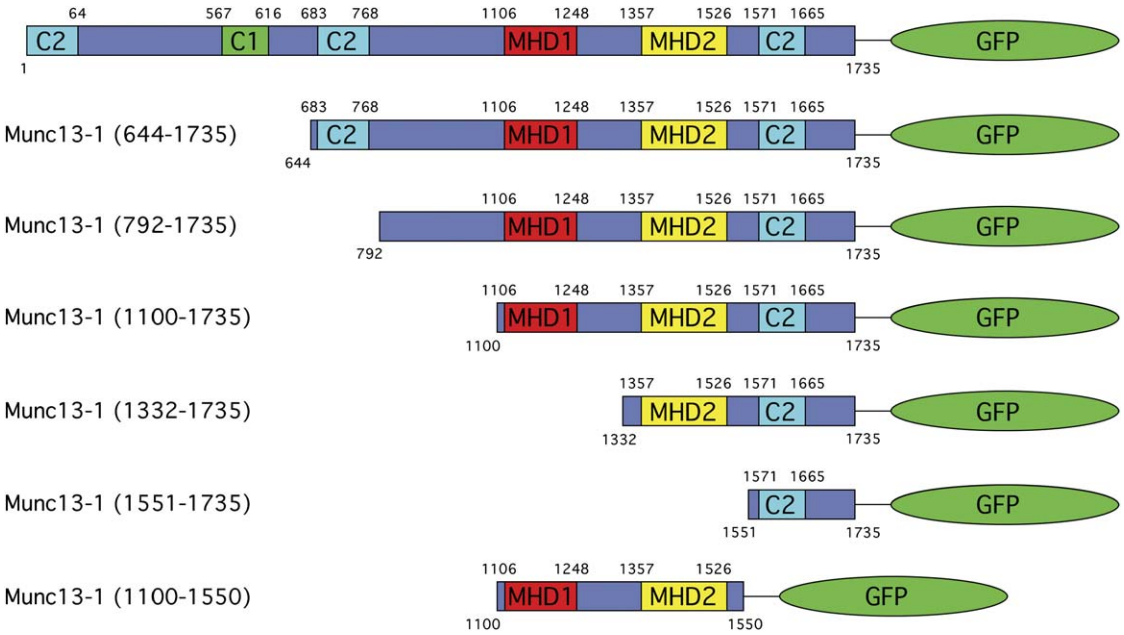


Figure 1. Munc13-1 Domain Structure and Deletion Constructs

Munc 13-1 contains three $C_2$ domains (aa 1–64, aa 683–768, and aa 1571–1665), one diacylglycerol binding and phorbol-ester binding $C_1$ domain (aa 567–616), and two Munc-homology domains (MHD, aa 1106–1248 and aa 1357–1526) [28, 29]. The domain structures of the deletion constructs used in this study are also shown, with the numbers referring to the residues of the originally published Munc13-1 sequence [28]. The green fluorescent protein (GFP) was fused to the C termini of Munc13-1 constructs with a spacer of 12 amino acids.

function. In order to test whether the C-terminal $C_2$ domain is also required for priming activity, we generated a fusion construct lacking this domain. As shown in Figure 2F, secretion from cells overexpressing Munc13-1 (1100–1550)-EGFP was again indistinguishable from that of untransfected control cells (103 ± 12 fF, n = 11, 128 ± 16 fF, n = 17, control), indicating that the presence of this C-terminal $C_2$ domain is also required for Munc13-1 priming activity. To rule out that the presence of the 27.4 kDa EGFP-tag immediately adjacent to the second MHD domain interferes with the accurate folding or the priming activity of the two MHDs, we also tested a construct in which the GFP protein was separated from the Munc13-1(1100–1550) sequence by an internal ribosome entry site (IRES), which will truncate translation of Munc13-1 and reinitiate translation of GFP. Because secretion in these cells was identical to that from control cells (data not shown), we conclude that the minimal region of Munc13-1 required for priming consists of the two MHDs and the C-terminal $C_2$ domain, i.e., Munc13-1 (1100–1735).

$Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels resulting from membrane depolarization (Figure 2) elicits a $Ca^{2+}$ signal of very complex spatial distribution, including so-called $Ca^{2+}$ microdomains [16]. As a result, the actual [$Ca^{2+}$] at a release site during a stimulus is not known. In order to investigate possible changes in the $Ca^{2+}$-dependent kinetics of exocytosis or granule recruitment, we stimulated control cells and cells overexpressing different deletion constructs by using flash-photolysis of the photolabile $Ca^{2+}$ chelator NP-EGTA. This procedure, which increased the intracellular [$Ca^{2+}$] in a step-wise manner and allowed accurate determination of the postflash [$Ca^{2+}$] (about 15–20 μM) by $Ca^{2+}$ indicators loaded through the patch pipet, resulted in stronger secretion. Flash photolysis of chromaffin cells expressing Munc13-1(644–1735)-EGFP resulted in an increase in both capacitance and amperometry responses as compared to control cells (RRP, 241 ± 36 fF versus 145 ± 21 fF; SRP, 237 ± 28 fF versus 167 ± 23 fF, $p < 0.05$; Figure 3A), consistent with our findings of the depolarization experiments (Figure 2). Importantly, the time constants of granule fusion from the releasable pools (SRP and RRP) were unchanged, indicating that active Munc13-1 deletion mutants selectively increase the size of the releasable granule pools without changing the kinetics of fusion. Furthermore, both size and slope of the sustained release component were identical between both groups, arguing that the $Ca^{2+}$-dependence of granule recruitment was also not altered by the active Munc13-1(644–1735)-EGFP deletion construct. Similar increases in SRP and RRP size were also obtained upon expression of Munc13-1(792–1735)-EGFP (RRP, 252 ± 26 fF versus 141 ± 24 fF; SRP, 201 ± 22 fF versus 157 ± 27 fF, $p < 0.05$; Figure 3B) and Munc13-1(1100–1735)-EGFP (RRP, 231 ± 37 fF versus 154 ± 31 fF; SRP, 191 ± 21 fF versus 145 ± 29 fF, $p < 0.05$; Figure 3C), whereas, as expected, expression of the C-terminal $C_2$ domain alone did not lead to a change in secretion as compared to control cells (Figure 3D). We conclude that the active Munc13-1 deletion constructs selectively increase the size of the releasable granule pools (SRP and RRP) without changing the $Ca^{2+}$ dependence of fusion or granule recruitment.

So far, our data show that the minimal region required for the priming function of Munc13-1 consists of the two MHDs and the C-terminal $C_2$ domain, i.e., Munc13-1

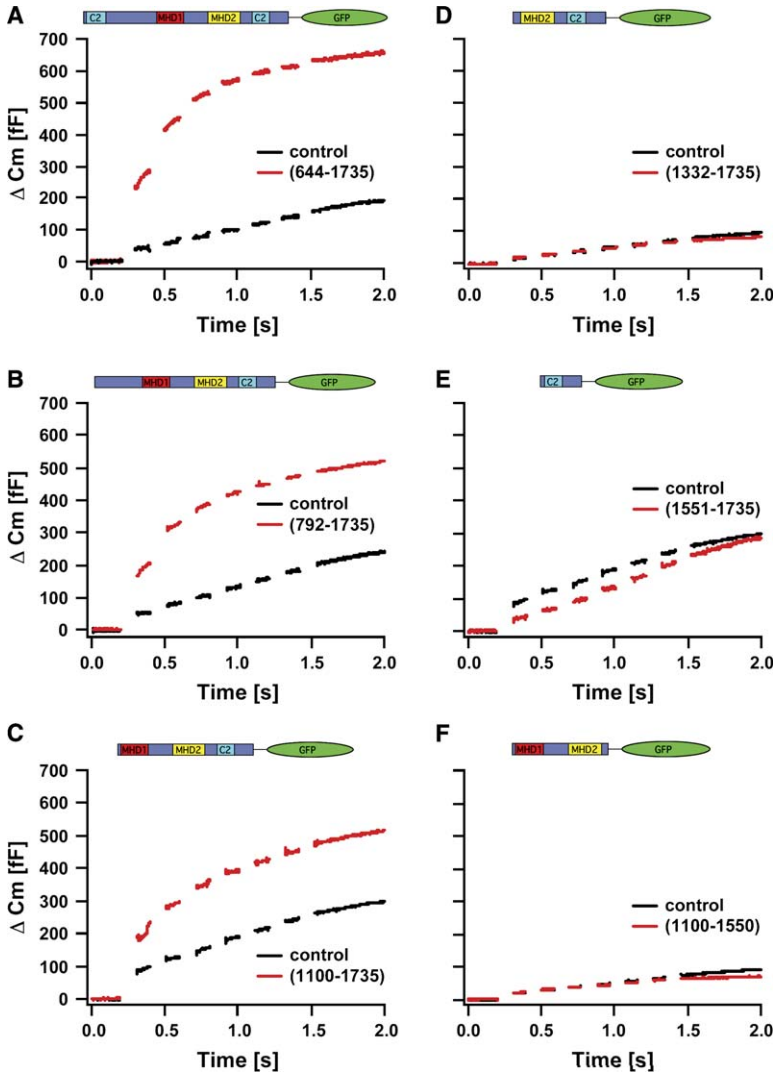


Figure 2. Depolarization-Induced Secretion in Chromaffin Cells Expressing Munc13-1 Deletion Constructs

Chromaffin cells expressing Munc13-1(644–1735)-EGFP (A), Munc13-1(792–1735)-EGFP (B), Munc13-1(1100–1735)-EGFP (C), Munc13-1(1332–1735)-EGFP (D), Munc13-1(1551–1735)-EGFP (E), and Munc13-1(1100–1550)-EGFP (F) were stimulated by seven 100 ms depolarizations at 10 Hz, and secretion was measured as increases in membrane capacitance. Whereas the constructs shown in (A–C) gave a robust gain-of-function effect, secretion from cells expressing constructs shown in (D–F) was indistinguishable from that in control cells. The domain structure of the deletion construct used is given on top of each panel.

(1100–1735). We found previously that Munc13-1 interacts through its MHDs with Syntaxin 1, a component of the SNARE complex, whose assembly most likely accounts for vesicle priming [17]. In order to determine whether the interaction of Munc13-1 with Syntaxin 1 is necessary for its priming function, we performed a yeast two-hybrid screen for mutants deficient in binding of Munc 13-1 to the autoinhibitory domain of Syntaxin (aa 1–79) (see Supplemental Experimental Procedures). We identified three different Syntaxin-1-binding-deficient missense mutants that all carried mutations within the minimal sequence required for priming. Whereas two mutants had single point mutations in MHD2 (I1364F) and the C-terminal $C_2$ domain (V1603D), respectively, the third mutant had three point mutations, one in MHD1, one in the linker sequence between MHD1 and MHD2, and one in the C-terminal $C_2$ domain (Q1190R/L1279P/D1655E). In addition, two point mutations in the MHD2 domain of the *C. elegans* Unc-13 protein (F1234A/K1236A) abolished the interaction with the autoinhibitory domain of Syntaxin (Madison et al., this issue of *Current Biology* [18]). We cloned Munc13-1(1100–1735) variants carrying these mutations into the pSFV expression vector and verified their expression in cultured kidney cells by western blotting (Figure 4A). Upon expression in chromaffin cells, none of these mutants caused an increase in secretion, whereas wild-type Munc13-1(1100–1735)-EGFP did (Figure 4B). The values for the individual point mutants were 152 ± 22 fF [Munc13-1(1100–1735)$^{V1603D}$-EGFP, n = 21], 159 ± 20 fF [Munc13-1(1100–1735)$^{Q1190R/L1279P/D1655E}$-EGFP, n = 15], 127 ± 25 [Munc13-1(1100–1735)$^{I1364F}$-EGFP, n = 12], and 120 ± 11 fF [Munc13-1(1100–1735)$^{F1234A/K1236A}$-EGFP, n = 15], whereas control cells and wild-type Munc13-1(1100–1735)-EGFP-expressing cells showed an average capacitance increase of 167 ± 14 fF (n = 44) and 359 ± 71 fF (n = 7), respectively. These data indicate that the direct interaction with Syntaxin 1 may be required for the priming function of Munc13-1 (see below).

## Discussion

In this study, we localized the protein sequence necessary for the priming function of Munc13-1 to the C-terminal third of the protein. This region, which contains both MHDs and the C-terminal $C_2$ domain, selectively increases the size of the slowly and readily releasable granule pools (SRP and RRP). Because the rates for

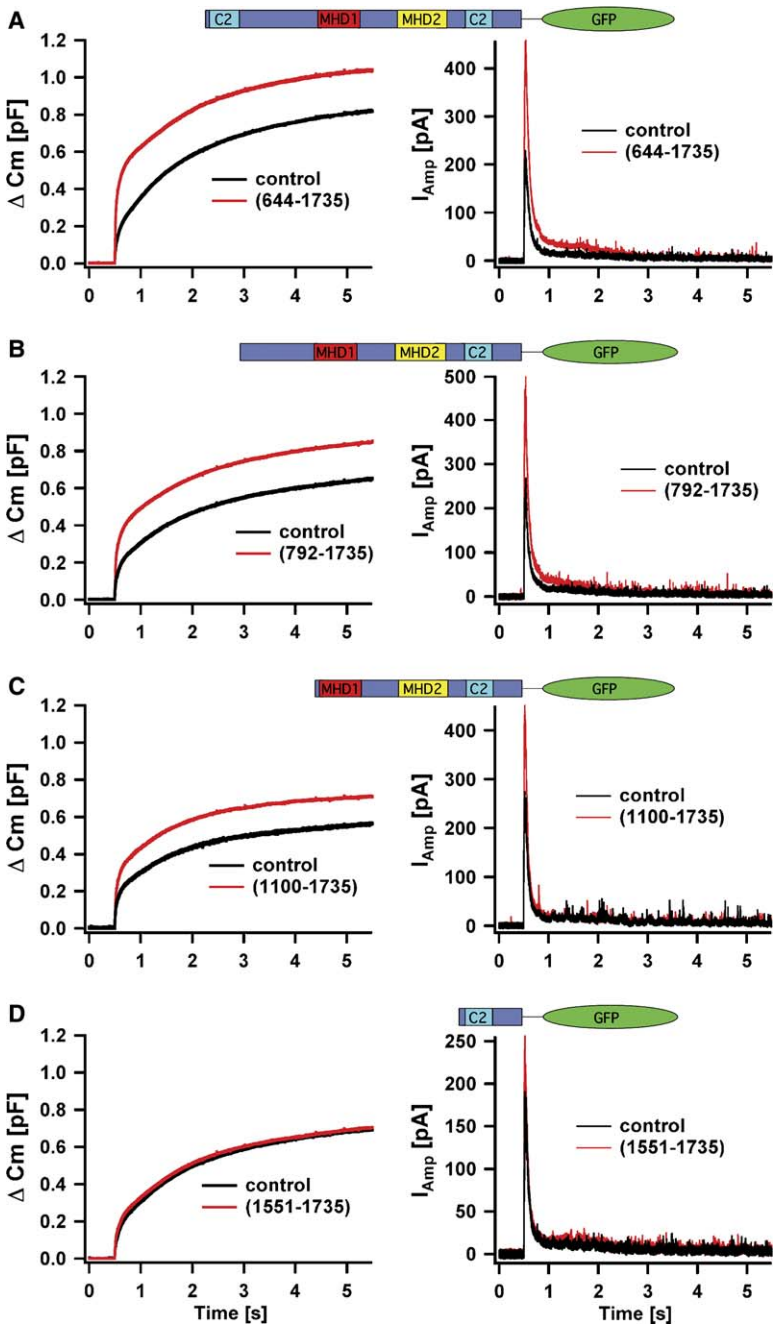


Figure 3. Secretion in Chromaffin Cells Expressing Munc13-1 Deletion Constructs Following Stimulation by Flash Photolysis of Caged $[Ca^{2+}]$

Chromaffin cells expressing Munc13-1(644–1735)-EGFP (A), Munc13-1(792–1735)-EGFP (B), Munc13-1(1100–1735)-EGFP (C), and Munc13-1(1551–1735)-EGFP (D) were stimulated by flash photolysis of $Ca^{2+}$-loaded NP-EGTA. The resulting secretion was captured by combined membrane capacitance (left panel) and amperometry measurements (right panel). All constructs containing both MHD domains and the C-terminal $C_2$ domain ([A–C], red traces) caused an increase in secretion as measured by membrane capacitance and amperometric current. The kinetics of secretion were indistinguishable from the respective control cells (black traces). In cells expressing Munc13-1(1551–1735)-EGFP, both amplitude and kinetics of secretion were identical to those in control cells (D). The domain structure of the deletion construct used is given on top of each panel.

priming and for fusion out of the SRP and RRP, which are all $Ca^{2+}$ dependent, are unaltered (Figure 3), we can also conclude that the action of Munc13-1 deletion mutants leaves the $Ca^{2+}$ dependence of fusion and granule recruitment unaffected. In addition, we show by using Syntaxin-1-binding-deficient point-mutant variants of Munc13-1 that the priming activity of Munc13-1 may require the direct interaction with Syntaxin 1.

Priming of vesicles represents a central step in $Ca^{2+}$-dependent exocytosis because only primed vesicles are fusion competent and can respond to an increase in intracellular $[Ca^{2+}]$ by fusing with the plasma membrane, thus executing transmitter release. According to the present view, the assembly of Syntaxin 1, SNAP-25, and Synaptobrevin 2 into the SNARE complex represents the molecular event that underlies the priming process. The rate-limiting factor in the SNARE-complex assembly reaction appears to be the availability of Syntaxin 1, which exists in two different conformations. In the closed conformation, the N-terminal H(abc) domain folds onto the C-terminal H3 domain, rendering this helical domain inaccessible for SNARE-complex formation [19, 20]. In the open conformation, the H(abc) domain is not folded back onto the H3 domain, thus leaving the H3 domain free to interact with three $\alpha$ helices of SNAP-25 and Synaptobrevin 2 and to form the four-helix bundle of the SNARE complex. Munc18, a cytoplasmic protein of the Sec1/Munc18-like family, can bind

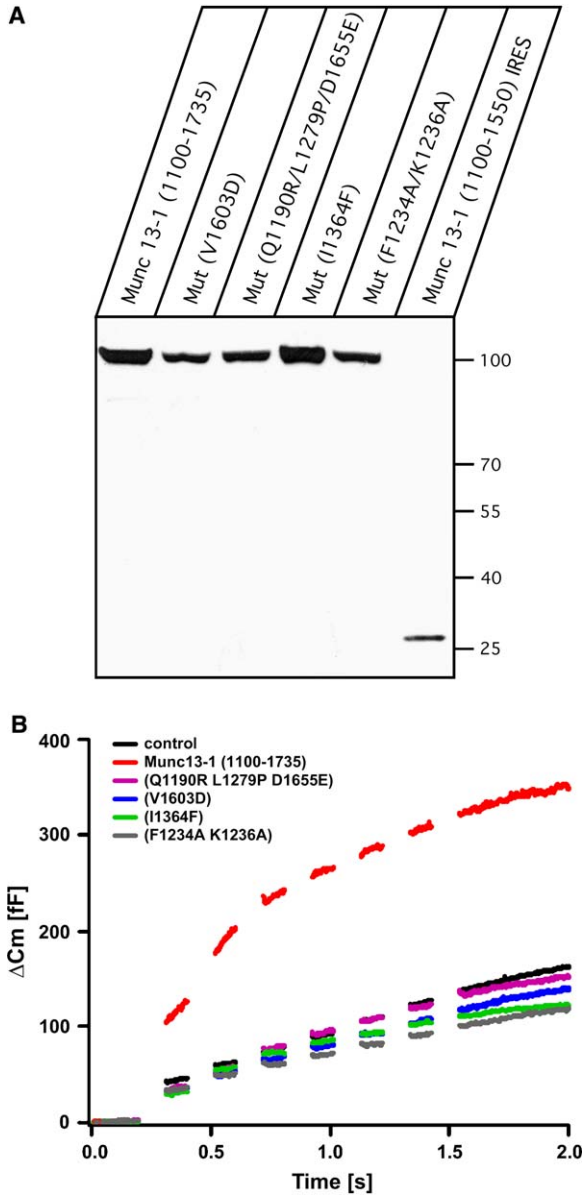


Figure 4. Depolarization-Induced Secretion in Chromaffin Cells Expressing Mutant Munc13-1 Variants Deficient in Syntaxin 1 Binding

(A) To test the expression level of the different Munc13-1 point mutants, we loaded 2.5 μg of total protein from BHK21 cells expressing the indicated constructs and analyzed them by western blotting with a monoclonal anti-GFP antibody. Relative molecular masses of marker proteins are given on the right.

(B) Chromaffin cells expressing Munc13-1(1100–1735) containing the mutations Q1190R/L1279P/D1655E (purple trace), V1603D (blue trace), I1364F (green trace), or F1234A/K1236A (gray trace), respectively, were stimulated by seven 100 ms depolarizations at 10 Hz. Secretion was measured as increases in membrane capacitance and compared to control cells (black trace) and cells expressing wild-type Munc13-1(1100–1735) (red trace). Whereas wild-type Munc13-1 (1100–1735) caused a gain of function in secretion, all Syntaxin 1 binding mutants showed secretion similar to that of control cells.

Syntaxin 1 in the closed conformation and may thereby inhibit SNARE-complex formation [20, 21]. Munc13-1 binds to the very N terminus of Syntaxin 1 [17], and biochemical experiments indicate that *C. elegans* Unc-13 may compete with Unc-18 for Syntaxin binding [22]. Our present study shows that a direct interaction of Munc13-1 with the autoinhibitory domain of Syntaxin 1 is required for Munc13-1 priming activity, supporting the hypothesis that Munc13-1-mediated vesicle priming involves its regulation of the Syntaxin 1 conformation [23]. The lack of priming activity in the Syntaxin-1-binding-deficient Munc13-1 mutants studied here is unlikely to be due to interference with mSec7-1- or β-spectrin binding of Munc13-1 [24, 25], which is also abolished by the introduced mutations, because, unlike Munc13-1, presynaptic spectrin regulates synapse stability but not the actual synaptic release process [26], and mSec7-1 has only mild regulatory effects on synaptic vesicle exocytosis, most likely by affecting synaptic vesicle recycling [27].

Our previous studies with yeast two-hybrid assays indicated that the Syntaxin 1 binding region of Munc13-1 is contained in residues 1181–1735 of Munc13-1 [17]. Further mapping of the binding site with cosedimentation assay revealed that a shorter fragment composed of residues 1181–1345 of Munc13-1 still bound Syntaxin 1, albeit with rather low affinity. According to the domain structure of Munc13-1 (Figure 1), residues 1181–1345 cover the C-terminal half of MHD1 and the region connecting MHD1 and MHD2. Our present electrophysiological data, on the other hand, show that even a larger construct containing MHD1, MHD2, and the linker region, Munc13-1(1100–1550) is unable to increase granule priming in adrenal chromaffin cells. Rather, for full priming activity in a physiological environment, the additional presence of the C-terminal $C_2$ domain of Munc13-1 is also required. One possible explanation for this finding is that the presence of the C-terminal $C_2$ domain supports the correct folding of the MHDs and thereby enables the efficient interaction with Syntaxin 1 in a cellular environment in vivo. In that case, the addition of sequences N terminal to the MHD1 instead of regions C terminal of MHD2 might also be sufficient to restore efficient Syntaxin 1 binding and full priming activity of MHDs 1 and 2. However, our western-blot analysis in cultured kidney cells (Figure S2) demonstrates that full-length Munc13-1(1100–1735) is expressed, and we have no evidence for improper folding of this construct. Furthermore, the fact that a single point mutation in the C-terminal $C_2$ domain (V1603D) abolishes Syntaxin 1 binding of Munc13-1 and its priming activity indicates an essential role of this $C_2$ domain in the binding to Syntaxin 1 and priming activity.

Whereas our results clearly demonstrate the essential role of the Munc13-1/Syntaxin interaction for vesicle priming, Madison et al. [18] show that in the neuromuscular junction of *C. elegans*, this interaction is also required for a postpriming step. Although we cannot rule out a contribution of other Munc13 isoforms to a postpriming action, it seems more plausible that these differences arise from the different systems, preparations, and techniques used in both studies, e.g., from the different definition of release-ready pools by either sucrose application or capacitance response to flash photolysis of caged $Ca^{2+}$. Further technical improvements might resolve this difference in the future, but both studies clearly demonstrate the functional importance of the Munc13/Syntaxin interaction for $Ca^{2+}$-dependent exocytosis.

## Conclusions

In this study, we define the minimal Munc13-1 domain that is responsible for its priming activity. Furthermore, by using point mutations deficient in Syntaxin 1 binding, we demonstrate that priming of secretory granules by Munc13-1 requires interaction with the SNARE component Syntaxin 1.

### Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and two Supplemental Figures and are available with this article online at: http://www.current-biology.com/cgi/content/full/15/24/2243/DC1/.

### Acknowledgments

We thank Dieter Bruns for stimulating discussions; Detlef Hof for help with data analysis; Josh Kaplan for sharing unpublished data; and Fritz Benseler, Carolin Bick, Ulrike Klemstein, Manuela Schneider, Ivonne Thanhäuser, and Reiko Trautmann for expert technical assistance. This work was supported by local funding ([HOMFOR, Univ. Saarland] to U.M. and J.R.), by the Max-Planck-Society (to N.B.), and by grants from the Deutsche Forschungsgemeinschaft (SFB406/A1 to N.B. and SFB530/C9 to J.R.).

Received: August 17, 2005
Revised: October 8, 2005
Accepted: October 25, 2005
Published online: November 3, 2005

### References

1. Südhof, T.C. (1995). The synaptic vesicle cycle: A cascade of protein-protein interactions. Nature *375*, 645–653.
2. Rettig, J., and Neher, E. (2002). Emerging roles of presynaptic proteins in Ca++-triggered exocytosis. Science *298*, 781–785.
3. Burgoyne, R.D., and Morgan, A. (2003). Secretory granule exocytosis. Physiol. Rev. *83*, 581–632.
4. Jahn, R., Lang, T., and Südhof, T.C. (2003). Membrane fusion. Cell *112*, 519–533.
5. Südhof, T.C. (2004). The synaptic vesicle cycle. Annu. Rev. Neurosci. *27*, 509–547.
6. Fernandez-Chacon, R., Königstorfer, A., Gerber, S.H., Garcia, J., Matos, M.F., Stevens, C.F., Brose, N., Rizo, J., Rosenmund, C., and Südhof, T.C. (2001). Synaptotagmin I functions as a calcium regulator of release probability. Nature *410*, 41–49.
7. Gundelfinger, E.D., Kessels, M.M., and Qualmann, B. (2003). Temporal and spatial coordination of exocytosis and endocytosis. Nat. Rev. Mol. Cell Biol. *4*, 127–139.
8. Brose, N., Rosenmund, C., and Rettig, J. (2000). Regulation of transmitter release by Unc-13 and its homologues. Curr. Opin. Neurobiol. *10*, 303–311.
9. Koch, H., Hofmann, K., and Brose, N. (2000). Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. Biochem. J. *349*, 247–253.
10. Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71–94.
11. Augustin, I., Rosenmund, C., Südhof, T.C., and Brose, N. (1999). Munc13–1 is essential for fusion competence of glutamatergic synaptic vesicles. Nature *400*, 457–461.
12. Richmond, J.E., Davis, W.S., and Jorgensen, E.M. (1999). UNC-13 is required for synaptic vesicle fusion in C. elegans. Nat. Neurosci. *2*, 959–964.
13. Aravamudan, B., Fergestad, T., Davis, W.S., Rodesch, C.K., and Broadie, K. (1999). Drosophila UNC-13 is essential for synaptic transmission. Nat. Neurosci. *2*, 965–971.
14. Ashery, U., Varoqueaux, F., Voets, T., Betz, A., Thakur, P., Koch, H., Neher, E., Brose, N., and Rettig, J. (2000). Munc13–1 acts as a priming factor for large dense-core vesicles in bovine chromaffin cells. EMBO J. *19*, 3586–3596.
15. Betz, A., Thakur, P., Junge, H.J., Ashery, U., Rhee, J.S., Scheuss, V., Rosenmund, C., Rettig, J., and Brose, N. (2001). Functional interaction of the active zone proteins Munc13–1 and RIM1 in synaptic vesicle priming. Neuron *30*, 183–196.
16. Neher, E. (1998). Vesicle pools and Ca2+ microdomains: New tools for understanding their roles in neurotransmitter release. Neuron *20*, 389–399.
17. Betz, A., Okamoto, M., Benseler, F., and Brose, N. (1997). Direct interaction of the rat unc-13 homologue Munc13–1 with the N terminus of syntaxin. J. Biol. Chem. *272*, 2520–2526.
18. Madison, J.M., Nurrish, S., and Kaplan, J.M. (2005). UNC-13 interaction with Syntaxin is required for synaptic transmission. Curr. Biol. *15*, this issue, 2236–2242. Published online November 3, 2005. 10.1016/j.cub.2005.10.049.
19. Fernandez, I., Ubach, J., Dulubova, I., Zhang, X., Südhof, T.C., and Rizo, J. (1998). Three-dimensional structure of an evolutionarily conserved N-terminal domain of syntaxin 1A. Cell *94*, 841–849.
20. Dulubova, I., Sugita, S., Hill, S., Hosaka, M., Fernandez, I., Südhof, T.C., and Rizo, J. (1999). A conformational switch in syntaxin during exocytosis: Role of munc18. EMBO J. *18*, 4372–4382.
21. Dulubova, I., Yamaguchi, T., Arac, D., Li, H., Huryeva, I., Min, S.W., Rizo, J., and Südhof, T.C. (2003). Convergence and divergence in the mechanism of SNARE binding by Sec1/Munc18-like proteins. Proc. Natl. Acad. Sci. USA *100*, 32–37.
22. Sassa, T., Harada, S., Ogawa, H., Rand, J.B., Maruyama, I.N., and Hosono, R. (1999). Regulation of the UNC-18-Caenorhabditis elegans syntaxin complex by UNC-13. J. Neurosci. *19*, 4772–4777.
23. Richmond, J.E., Weimer, R.W., and Jorgensen, E.M. (2001). An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming. Nature *412*, 338–341.
24. Neeb, A., Koch, H., Schürmann, A., and Brose, N. (1999). Direct interaction between the ARF-specific guanine nucleotide exchange factor msec7–1 and presynaptic Munc13–1. Eur. J. Cell Biol. *78*, 533–538.
25. Orita, S., Naito, A., Sakaguchi, G., Maeda, M., Igarashi, H., Sasaki, T., and Takai, Y. (1997). Physical and functional interactions of Doc2 and Munc13 in Ca2+-dependent exocytotic machinery. J. Biol. Chem. *272*, 16081–16084.
26. Pielage, J., Fetter, R.D., and Davis, G.W. (2005). Presynaptic spectrin is essential for synapse stabilization. Curr. Biol. *15*, 918–928.
27. Ashery, U., Koch, H., Scheuss, V., Brose, N., and Rettig, J. (1999). A presynaptic role for the ADP ribosylation factor (ARF)-specific GDP/GTP exchange factor msec7–1. Proc. Natl. Acad. Sci. USA *96*, 1094–1099.
28. Brose, N., Hofmann, K., Hata, Y., and Südhof, T.C. (1995). Mammalian homologues of Caenorhabditis elegans unc-13 gene define novel family of C2-domain proteins. J. Biol. Chem. *270*, 25273–25280.
29. Betz, A., Telemenakis, I., Hofmann, K., and Brose, N. (1996). Mammalian Unc-13 homologues as possible regulators of neurotransmitter release. Biochem. Soc. Trans. *24*, 661–666.